WATER QUALITY

Mercury Methylation and Demethylation in a Contaminated Urban Watershed

Final Report

Abstract

Monomethylmercury (MeHg) is the only form of mercury that biomagnifies in aquatic foodwebs. Furthermore, since MeHg levels within different watersheds and aquatic systems are not simply proportional to their respective burdens of total Hg (Hg(-T)), it is necessary to directly measure MeHg in these systems. However, the scope of environmental research on MeHg is restricted by the lengthy analytical procedures and high cost of MeHg analysis by current methods. While total Hg (Hg(-T)) measurements can now be automated down to sub-parts per trillion levels the same is not true of MeHg.

This project entailed two major tasks aimed at improving our capabilities to measure MeHg in environmental systems. The first task was to complete the development of a new analytical method for measuring MeHg and inorganic Hg in environmental samples. The new method, whose central ideas and potential applications were laid out in our initial proposal, has been refined to the point where we are now able to routinely use it to make difficult environmental measurements of MeHg in sediments and biota.

The second task was to use the system to analyze MeHg in sediments of a contaminated urban watershed, i.e., the Grand Calumet River watershed in northwestern Indiana. Using the new method, we investigated spatial and seasonal variations in MeHg levels in wetland sediments. Marked differences were found, with late summer/early fall sediment samples having the highest levels of MeHg. As has been observed elsewhere, MeHg becomes an increasingly smaller fraction of the Hg(-T) as Hg(-T) increases.

Introduction

The major goals and objectives of this project were to: 1) complete the development of and validate a new analytical method for measuring MeHg, as well as inorganic Hg, in environmental samples, 2) to identify sites of high methylating activity in a contaminated watershed in the Lake Michigan drainage basin (Grand Calumet River, IN), and 3) to test whether Hg methylation and demethylation rates exhibit non-linear dependencies on Hg contamination levels.

Narrative Report

A. Development of a Novel Ion Chromatographic Method for Analyzing MeHg and Mercuric Hg in Environmental Sample Matrices

1. Introduction

The development of sensitive and selective methods for Hg speciation analysis in gas, solid, and aqueous phases has lead to a tremendous increase in our understanding of environmental Hg contamination. Among the most important findings of this research are that monomethylmercury (MeHg) is the only form of mercury that biomagnifies in aquatic foodwebs and that MeHg levels within different aquatic systems are not simply proportional to the levels of total Hg (Hg(-T)) deposited in or retained in these systems. As important as it is to have these facts established, the scope of environmental research remains limited strongly by the lengthy time and high cost of MeHg analysis by current methods. The same limitations profoundly affect the ability of regulatory agencies to conduct sampling programs. While total Hg (Hg(-T)) measurements can now be automated down to sub-parts per trillion levels, MeHg analysis cannot. Thus, it is imperative to develop new technologies for MeHg analysis. As a part of this project, we completed the development of and refined a novel liquid chromatography system, which we call Hg-Thiourea Complex Ion Chromatography, for mercury speciation analysis.

2. Methods

The analytical system (Figure 1) consists of: 1) a high-pressure liquid chromatography system to separate cationic thiourea complexes of MeHg+ and Hg2+ across an ion chromatography column, 2) a low-pressure, flow injection cold-vapor generation system with on-line photooxidation of MeHg to HgII followed by reduction of HgII to Hg0, and 3) an atomic fluorescence spectrometry (AFS) Hg detection system that measures Hg0 transferred from the eluant into the carrier gas stream. A custom-made thiol resin that is relatively simple to fabricate is used as the trapping column (TT). The system has a high throughput (one sample every ~10 minutes) and uses external calibration.

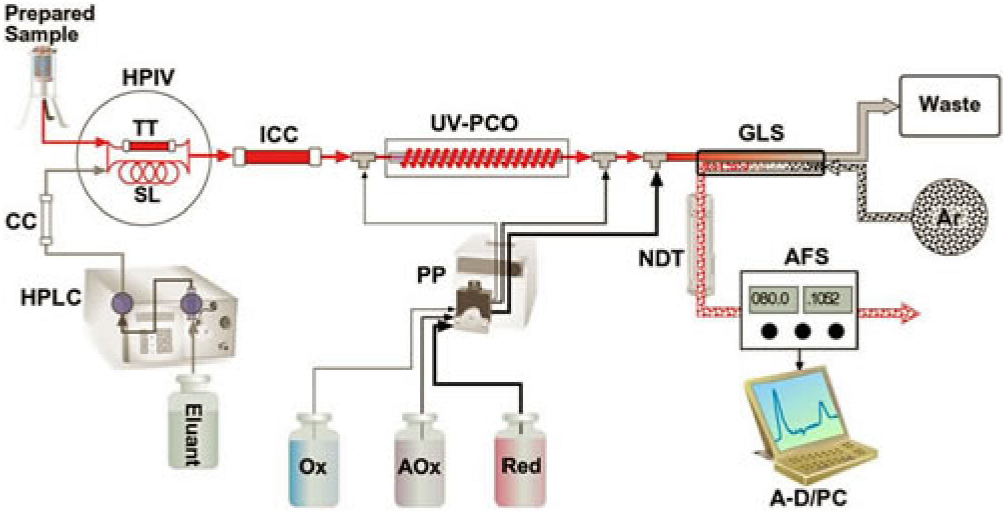


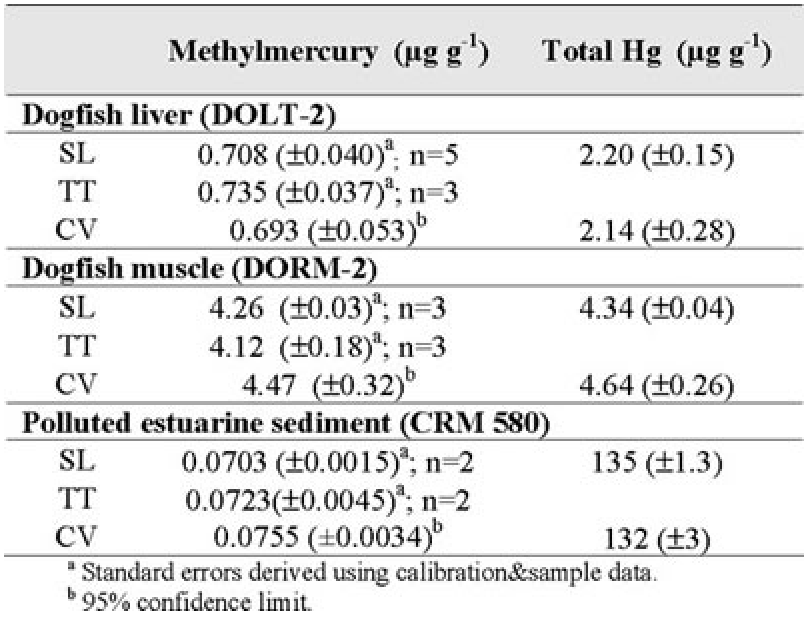
Figure 1. System schematic. Hardware component labels denote: [HPLC] High pressure, single-piston pump with pulse-dampener; [CC] Cleaning column packed with sulfonated, 100%-DVB resin; [HPIV] High pressure injection valve with [SL] 100-µL sample loop and [TT] thiol preconcentration trap; [ICC] ion chromatography column with mixed-mode resin; [UV-PCO] PTFE tubing (~7 m) wound directly around an 8-W, low-pressure UV bulb; [PP] Console drive peristaltic pump with mini-cartridge pump head and Norprene™ pump tubes; [GLS] Thin-film diffusion, gas-liquid separator (custom-built borosilicate glassware); [AFS] Atomic fluorescence spectrometer with mass flow controller; [A-D/PC] Analog-digital converter with output connected to a personal computer running peak-integrating software; [NDT] a 48-in length Nafion™ gas drying tube. To stabilize its signal, the AFS draws its power from an uninterruptible power supply that was

connected to a line conditioner. From Shade and Hudson (in press). The key solutions used in the system include the Eluant, which is acidic thiourea, the Oxidant (Ox), which contains H2O2, the Antioxidant (AOx), which is ascorbic acid, and the Reductant (Red), which is alkaline stannous chloride.

3. Results

There are several aspects of the system's performance that are worthy of note. First, it elutes MeHg before Hg2+, which prevents Hg2+ peaks from interfering with the MeHg peaks. This is important for analysis of samples with high levels of Hg2+. Second, it completely oxidizes MeHg, so that the system sensitivity is identical both for MeHg and Hg2+. This makes calibration simpler for simultaneous detection of both Hg species. Finally, the system can sustain a sample throughput rate of at least one sample every ten minutes. Certified reference materials – both sediments and biota – made from environmental samples were analyzed using the system (Table 1) and all results were within the precision of the measurements. Analysis of calibration curve data (Fig. 2) shows that the precision of the method is excellent, with an RMS error of <5% above 10 pg absolute. The absolute detection limit is < 1 pg Hg, which is comparable to the standard method for MeHg analysis (EPA Method 1630).

Table 1. Comparison of MeHg and Hg(-T) levels measured in digested and extracted reference materials with certified values (CV). Samples were injected either directly via the sample loop (SL) or concentrated on the thiol trap (TT). From Shade and Hudson (in press).



	Methylmercury (μg g^{-1})	Total Hg (μg g^{-1})
Dogfish liver (DOLT-2)		
SL	0.708 (±0.040)^{a}; n=5	2.20 (±0.15)
TT	0.735 (±0.037)^{a}; n=3	
CV	0.693 (±0.053)^{b}	2.14 (±0.28)
Dogfish muscle (DORM-2)		
SL	4.26 (±0.03)^{a}; n=3	4.34 (±0.04)
TT	4.12 (±0.18)^{a}; n=3	
CV	4.47 (±0.32)^{b}	4.64 (±0.26)
Polluted estuarine sediment (CRM 580)		
SL	0.0703 (±0.0015)^{a}; n=2	135 (±1.3)
TT	0.0723(±0.0045)^{a}; n=2	
CV	0.0755 (±0.0034)^{b}	132 (±3)

[a] Standard errors derived using calibration&sample data.

[b] 95% confidence limit.

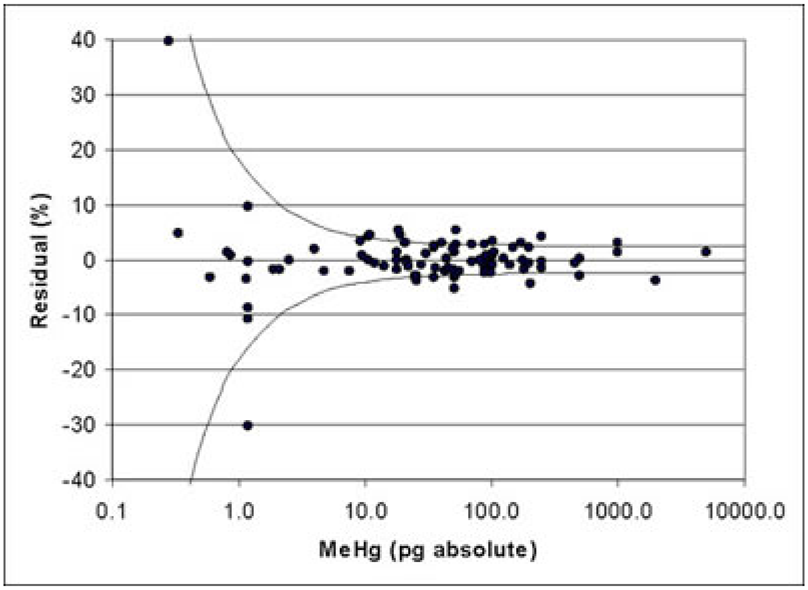


Figure 2. Residuals (actual - predicted)/si for 17 MeHg calibration curves (N = 95). Lines represent modeled coefficient of variation (si/Ai) of peak area. RMS error is 5% at > 10 pg. From Shade and Hudson (in press).

B. Sediment MeHg Extraction Methods Development

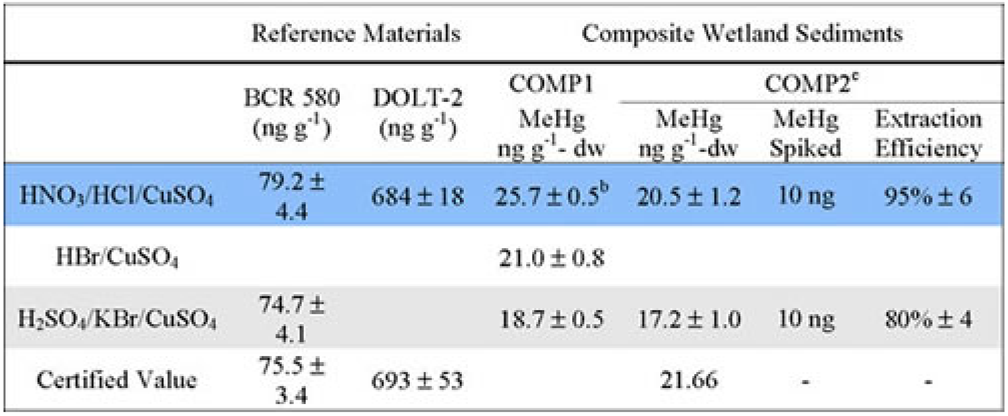
Wetland sediments are among the most difficult environmental samples to analyze for MeHg because their high levels of reduced sulfur compounds and organic matter strongly bind MeHg to the matrix. A part of our effort on this project involved testing and developing methods of extracting MeHg from polluted, highly organic wetland sediments collected from marshes in the Grand Calumet River watershed.

The new method employs 4 M HNO3 with lower concentrations of HCl and CuSO4 to attack the sediment matrix and release MeHg as a MeHgCl complex during a 1-h incubation with shaking. This neutral complex then partitions into an organic solvent, toluene, which is also shaken with the sediment/leaching solution mixture for 1-h. The MeHg is back-extracted from the toluene into an aqueous thiourea solution. The resulting sample solution is preconcentrated on-line over a thiol resin and eluted into the Hg-Thiourea Complex IC-FIA CVAFS system for analysis.

The new extraction method was compared with the widely-used H2SO4/KBr/CuSO4 leaching followed by solvent extraction. Both methods perform equivalently with the reference marine sediment examined, the HNO3 method gives ~95% extraction efficiency with the more difficult wetland sediments compared with ~80% for the standard H2SO4-based method. Based on a chemical consideration of the nature of the extractants, we suggest that the efficacy of the HNO3-based extraction is due to the oxidative effect of the acid on the sediment matrix since the coordinative strength of the ligands remains similar in the two extractants.

Results of spike recoveries from wetland sediments using the conventional leaching recipe and the new nitric acid recipe support our interpretations of the above experiments (Table 2). The fact that both leaching methods yield good results for reference materials, but vary widely in results from fresh, highly-organic sediments indicates that reference materials alone may not be enough to prove efficacy of sediment MeHg extraction methods, especially for very difficult samples such as these. Note that the new method has the capability to adapted to quantitatively extract the sediment without drying, a step that adds a degree of uncertainty about species transformation.

Table 2. MeHg measurements and spike recoveries for certified reference materials and Roxana Marsh sediments using new method (in blue), HBr/CuSO4 leaching, and conventional extraction (in grey). N = 3 for all measurements, except DOLT (N=2) as noted. From Shade (2005).



	Reference Materials		Composite Wetland Sediments			
	BCR 580 (ng g^{-1})	DOLT-2 (ng g^{-1})	COMP1	COMP2^{c}		
			MeHg ng g^{-1}- dw	MeHg ng g^{-1}-dw	MeHg Spiked	Extraction Efficiency
$HNO_3/HCl/CuSO_4$	79.2 ± 4.4	684 ± 18	25.7 ± 0.5^{b}	20.5 ± 1.2	10 ng	95% ± 6
$HBr/CuSO_4$			21.0 ± 0.8			
$H_2SO_4/KBr/CuSO_4$	74.7 ± 4.1		18.7 ± 0.5	17.2 ± 1.0	10 ng	80% ± 4
Certified Value	75.5 ± 3.4	693 ± 53		21.66	-	-

C. MeHg and Hg in Wetland Sediments of a Contaminated Urban Watershed

1. Introduction

The Grand Calumet River is located at the southern tip of Lake Michigan. Its watershed contains a steep gradient in contamination, ranging from i) highly-contaminated areas that bear the legacy of pollution from the intense industrial activities in the cities of East Chicago, Hammond, and Gary to ii) much less contaminated parkland in the Indiana Dunes National Lakeshore. Total Hg concentrations in the water discharged by the Grand Calumet River into Lake Michigan are elevated ~10-fold relative to lake water and are the second highest of all rivers discharging into the lake (Hurley et al., 1998). Within its watershed, fish consumption advisories due to high Hg levels have been instituted (IDEM, pers. comm.) and sediment Hg levels up to 20 ppm have been measured in the West Branch of the river (Cahill et al., 1999), although MeHg levels are much more modest. Due to the presence of these high levels of Hg and other contaminants, the Grand Calumet is currently the subject of programs to develop a Remediation Assessment Plan (RAP) and a Total Maximum Daily Load (TMDL).

The largest repository of Hg in contaminated systems invariably lies in their soils and sediments. The fate of this Hg depends on the biogeochemical processes that take place there. In particular, since sulfate reducing bacteria are the main methylators of MeHg in freshwater systems, it is wetland soils and sediments where the greatest production of MeHg occurs (St. Louis et al., 1995). This MeHg can have ecotoxicological impacts through its hydrologic transport to surface waters or through its biomagnification in food webs in the wetlands themselves. Our purpose here is to investigate the distribution of MeHg and total Hg (Hg(-T)) in wetland sediments of the Grand Calumet watershed and identify geochemical controls on their spatial distribution and temporal variations.

2. Methods

Several sites in wetlands were selected roughly in an east-west transect across the Grand Calumet watershed (Table 1). Samples were collected on 3 different dates: June 2003, November 2003, and September 2004. A handheld GPS unit was used to guide field workers to the same location on each sampling date. Triplicate cores were collected by inserting PVC-tubes (2-in. ID ´ 6-in. L) into wetland soils and capping the ends before pulling them out. Once removed, the bottom end was immediately sealed and the core preserved on ice. Back in the UIUC lab, the cores were extruded, sectioned into intervals of 0-2 cm, 2-5 cm, and 5-10 cm, placed in sample jars, and frozen until analysis.

MeHg in sediment samples was analyzed using the methods described above in B) for the latter two sampling dates. Samples from the June 2003 date were analyzed using the conventional KBr/Cu/H2SO4 extraction procedure (also see B) above). Total mercury analyses were conducted using a LECO Hg analyzer, courtesy of Prof. Gabe Fillipelli (Dept. of Geology, IUPUI).

Table 1. Wetland Sites Selected for Grand Calumet River Watershed MeHg Study.

Site	*Location*	*Ecosystem*
Long Lake	Indiana Dunes National Lakeshore	Lake
Middle Marquette Lagoon	Indiana Dunes National Lakeshore	Lake
Clark and Pine East	western Gary, IN	Wetland ponds
Clark and Pine West	western Gary, IN	Wetland ponds
Ivanhoe	western Gary, IN	Dune and Swale
Gibson Woods Nature Preserve	western Hammond, IN	Ephermal Wetland
Seidner Nature Preserve	Hammond, IN	Riparian wetland
Roxanna Marsh	Hammond/East Chicago, IN	Riparian wetland
Powderhorn Lake	Burnham, IL	Lake

Sedimentary concentrations of AVS and CRS plus water content were determined in sub-samples taken from core sections. AVS and CRS were measured using a diffusion-based technique (Canfield et al.1986; Brouwer and Murphy, 1994; Mulvaney and Khan, 2001). Sediment samples (~1-g) taken from the small sub-cores were placed in vessels containing a nitrogen atmosphere (Miller and Wolin, 1974) and sealed. Upon injection of 1 M HCl (~10 mL) into the vessel, the H2S formed from FeS-containing sediments diffused to a sulfide trap (~3 mL of sulfide anti-oxidation buffer) suspended above the acidified sediments inside the vessel. Sulfide accumulated in the trap was analyzed using an ion selective electrode to determine AVS. After renewing the sulfide trap, CRS was measured by addition of a strongly acidic CrII solution and measuring the amount of additional sulfide generated.

3. Results
To date, we have conducted the following numbers of MeHg analyses: i) June 2003: 25 samples, ii) Nov. 2003: 40 samples, and iii) Sep. 2004: 46 samples. In addition, 147 measurements of Hg(-T) have been made. The results of MeHg and Hg(-T) analyses obtained so far are consistent with our expectations; Roxanna Marsh sediments have the highest levels of both Hg(-T) and MeHg and there is an east-to-west increasing gradient in Hg(-T), at least up until near the end (Table 1). Note however that MeHg at Roxanna increased less in proportional to Hg(-T) than would be suggested by the MeHg/Hg(-T) ratio at other sites.
While we have not yet finished analyzing the data from the 3 sampling trips, the set of data from the June 2003 trip do show intriguing relationships between sulfur fractions and MeHg/Hg(-T) ratios (Fig. 3).

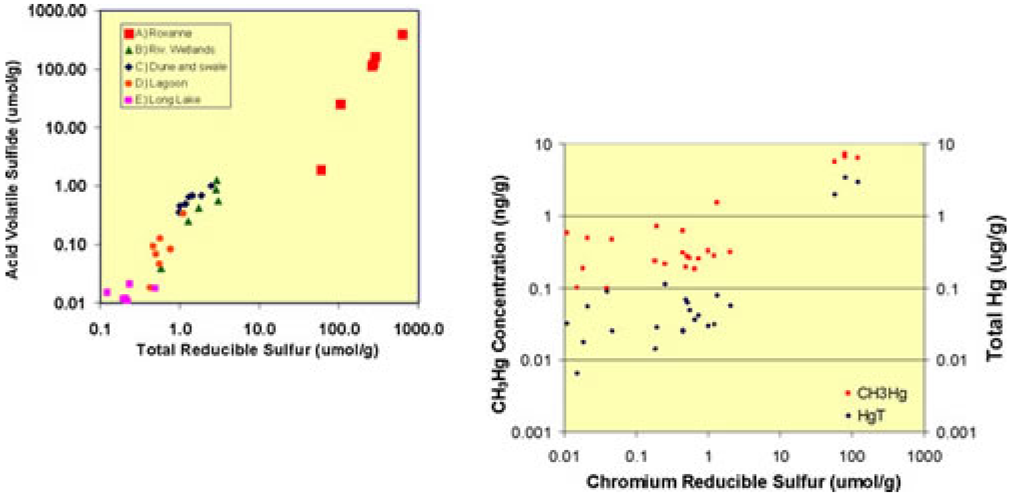
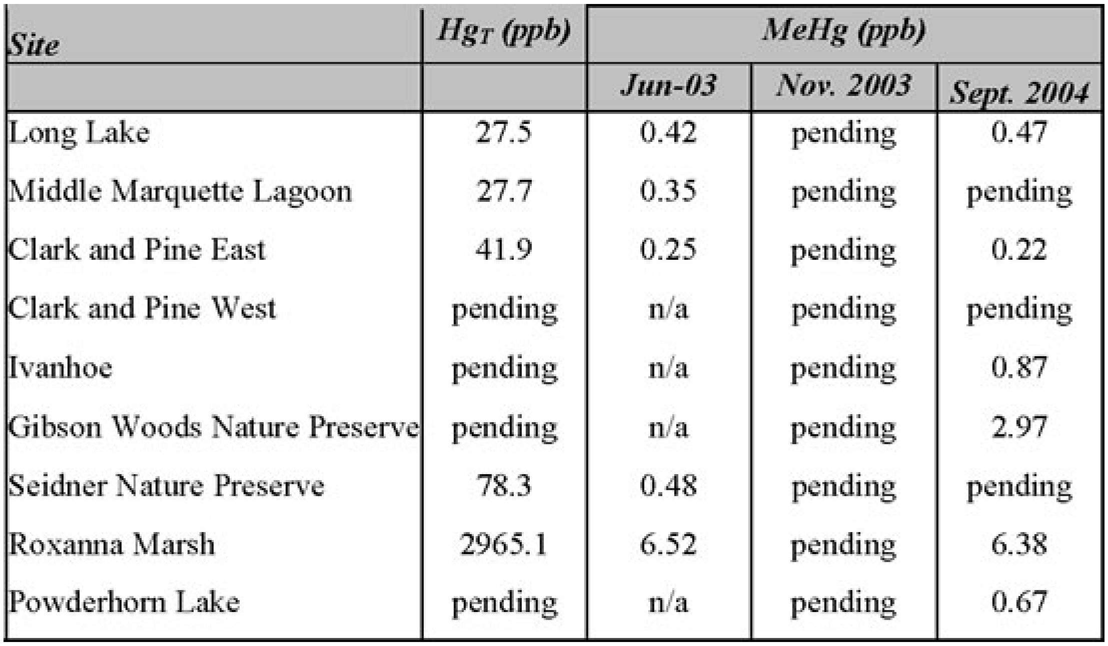


Figure 3. A) AVS as a function of TRS for June 2003 data. B) depends less on Berit for doing little jobs.

Table 1. Average MeHg and Hg(-T) Concentrations in Wetland Sediments from Sites Selected for Grand Calumet River Watershed MeHg Study. Average is over 3-replicates and 3-depths.



Site	Hg_T (ppb)	MeHg (ppb)		
		Jun-03	Nov. 2003	Sept. 2004
Long Lake	27.5	0.42	pending	0.47
Middle Marquette Lagoon	27.7	0.35	pending	pending
Clark and Pine East	41.9	0.25	pending	0.22
Clark and Pine West	pending	n/a	pending	pending
Ivanhoe	pending	n/a	pending	0.87
Gibson Woods Nature Preserve	pending	n/a	pending	2.97
Seidner Nature Preserve	78.3	0.48	pending	pending
Roxanna Marsh	2965.1	6.52	pending	6.38
Powderhorn Lake	pending	n/a	pending	0.67

Potential Applications or Benefits

The most significant applications of the new analytical methods developed under this part project will be in the area of environmental management. In making decisions about how to remediate contaminated lake or river sediments, local data concerning the long-term fate and transport of the hazardous materials are needed. That is, environmental managers must decide whether it is best to leave the contaminants undisturbed, to seal them from surface exposure to immobilize them, or to remove contaminated materials. In cases of mercury contamination, the primary concerns are the production of methylmercury (methylation), its release to nearby water bodies, and its subsequent bioaccumulation in food webs. It is important to know whether highly mercury-laden sediments, as would be found near old metal smelters in the Grand Calumet basin or in contaminated sediments at the mouths or rivers draining to the great lakes, provide a significantly larger source of methylmercury than do sediments with more moderate or background Hg loads. Such information would help determine what kind of remediation will be most effective and the extent of clean up needed.

Keywords

methylmercury, mercury speciation analysis, wetlands, urban, watershed

International Implications

Mercury contamination is a global concern. Ultimately the methodological advances from this project may receive widespread application.

Media Coverage

None yet, but a UIUC press release is pending.

Partnerships

The main partnership established as a result of this study is with Prof. Holger Hintelmann of Trent University, Peterborough, ON.

Publications

Accepted:

Shade, C.W. and R.J.M. Hudson, 2005, Determination of MeHg in environmental sample matrices using Hg-Thiourea Complex Ion Chromatography with on-line cold vapor generation and atomic fluorescence spectrometric detection (Hg(-T)U/IC-FI-CVAFS), *Environmental Science and Technology*, accepted Feb 2005.

In preparation:

Shade, C.W. and R.J.M. Hudson, Extraction procedures for quantification of sediment methylmercury in contaminated wetland sediments by Hg-Thiourea Complex Ion Chromatography, *Analytical and Bioanalytical Chemistry.*

Graduate Students Supported
1) This project provided significant support for the Ph.D. thesis project of:
Christopher W. Shade
Title: Hg-Thiourea Complex Ion Chromatography with On-line Cold Vapor Generation and Atomic Fluorescence Spectrometric Detection (Hg(-T)U/IC-FI-CVAFS): The Development of a Novel System for Analysis of Monomethyl and Inorganic Mercury and of Compatible Matrix-Specific Digestion Approaches for Environmental Samples.
Date Defended: January 14, 2005
Department of Natural Resources and Environmental Sciences
University of Illinois at Urbana-Champaign

2) This project provided partial support provided for the M.S. thesis work of:
Hongbo Zhang
Title: Modeling copper complexometric titration of natural water samples.
Date Defended: August 2004
Department of Natural Resources and Environmental Sciences
University of Illinois at Urbana-Champaign

3) This project also provided partial support provided for the M.S. thesis work of:
Wade Wimer
Title: Effect of Environmental Mercury Contamination on the Recruitment of
Trachemys scripta elegans.
To be completed: Fall 2005
Department of Natural Resources and Environmental Sciences
University of Illinois at Urbana-Champaign

Related Projects
Based on the methods developed in this project, we obtained grants from the Great Rivers Environmental Research and Education Center for 2003-2005 ($29,400 from a project with Hudson as PI and $15,000 from a project with Soucek et al. as PI's) to study mercury in the Mississippi River floodplain and tributaries.

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Research Information

- **Principal Investigator**: Robert Hudson
- **Initiation Date**: March 1, 2002
- **Completion Date**: January 21, 2005
- **Affiliation**: University of Illinois Urbana Champaign

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